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Effects of Chromium (VI) and Humic Substances on Selected Physiological Responses of *Azolla caroliniana*

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ABSTRACT.—The independent and interactive effects of Cr(VI) and humic substances were investigated on several physiological and biochemical responses of *Azolla caroliniana*. Cr(VI) concentrations of 1 and 2 mg l^{-1} were found to cause a reduction in growth and chlorophyll *a* and *b* concentrations of *Azolla* with and without the coexistence of humic substances. The Cr(VI) toxicity on these parameters was less pronounced in the presence of humic substances. Carotenoid concentrations were not significantly affected by Cr(VI) treatments while humic substances, in the absence of Cr(VI), influenced an increase in carotenoid concentration. Anthocyanins were increased in treatments receiving Cr(VI) and humic substances, both individually and together. Increases in anthocyanins were less pronounced in treatments receiving humic substances with Cr(VI) in comparison to treatments receiving Cr(VI) in the absence of humic substances. Cr(VI) and humic substances influenced an increase in sucrose, starch, and total nonstructural carbohydrate (TNC) accumulation. In the coexistence of Cr(VI) and humic substances, accumulation of carbohydrates was reduced in comparison to those receiving Cr(VI) in the absence of humic substances. Humic substances in the absence of Cr(VI) influenced a significant decrease in the activities of sucrose phosphate synthase (SPS) and invertase and a significant increase in the activity of sucrose synthase (SS). Cr(VI)was insignificantly influenced SPS activity at 1 mg l^{-1} and at 2 mg l^{-1}. Invertase activity was significantly increased at both Cr(VI) treatments. Humic substances and Cr(VI) interactively resulted in increased SS activity insignificantly above those treatments receiving Cr(VI) in the absence of humic substances. Phosphate synthase activity was reduced at both Cr(VI) treatments when humic substances were coexistent in comparison to treatments receiving Cr(VI) in the absence of humic substances. Invertase activities were reduced in treatments receiving Cr(VI) and humic substances together in comparison to those receiving Cr(VI) in the absence of humic substances.

Chromium (Cr) finds its way into aquatic ecosystems predominantly through industrial processes such as metallurgical operations, tanning, dyeing, electroplating, and paint manufacturing (Hartford, 1979). It has been estimated that approximately 1068 metric tons of chromium are released into the biosphere annually (Nriagu, 1988). Of the various forms of chromium, the most commonly occurring are the trivalent form, Cr(III), and the hexavalent form, Cr(VI). Of these, Cr(VI) is the most readily assimilated by biotic systems and is the most toxic form of chromium known (Schroll, 1978; Towill et al., 1978).

The effects of Cr have been studied on a relatively wide range of aquatic plants. In the angiosperm, *Myriophyllum spicatum* L. (Haloragaceae), it was shown that Cr(VI) concentrations up to 50 μ l^{-1} caused an increase in shoot

growth, whereas higher concentrations resulted in a nearly linear reduction in plant growth (Guilizzoni et al., 1984). Furthermore, Staves and Knaus (1985) showed that Cr(VI) concentrations exceeding 1.0 mg l^{-1} resulted in a decrease in growth rate of the angiosperm genera, *Lemna* and *Spirodela* (Lemnaceae). Sela et al. (1989) obtained comparable results with the fern, *Azolla filiculoides* Lam. (Azollaceae), with Cr(VI) concentrations ranging from 8 to 15 μg l^{-1} reducing growth rates. Similar results were obtained by Sarkar and Jana (1986) in that Cr(VI) concentrations of 2 and 5 mg l^{-1} resulted in a decreased growth rate as well as a reduction in chlorophyll concentration of *Azolla pinnata* R. Br. However, Cr(VI) concentrations of 0.02, 0.2, 1.1 and 2.2 mg l^{-1} were shown to influence an increase in photosynthetic rates of the angiosperm, *Nelumbo lutea* (Willd.) Pers. (Nelumbonaceae) after 48 hours incubation at a pH of 5.6 (Francko et al., 1993).

In nature, chromium most likely exists in the presence of decomposed organic matter. Terrestrially derived humic acid and aqueous humic acid were shown to reduce the effects of copper toxicity to the green alga *Chlamydomonas reinhardtii* P.A. Dang. (Garvey et al., 1991). These same investigators demonstrated that aqueous fulvic acid had little or no effect on copper toxicity to this green alga. Hongve et al. (1980) reported that humic substances had no effect on the cadmium toxicity to phytoplankton photosynthesis. However, there is very limited information available on the role of naturally extracted humic substances on the influence of heavy metals on aquatic plants in general, and especially the fern genus *Azolla*. The objective of this investigation was to determine the interactive effects of two concentrations of Cr(VI) (1.0 and 2.0 mg l^{-1}) and humic substances (extracted from leaves of *Typha latifolia* L. [Typhaceae]) on *Azolla caroliniana* Willd. growth and chlorophyll, carotenoid, and anthocyanin concentration. Humic substances extracted from *Typha latifolia* were used in other physiological and biochemical investigations of planktonic and attached algae, bacteria, and vascular aquatic plants (Kim and Wetzel, 1993; Wetzel, 1993). To understand the influence of the above growth conditions on the carbohydrate status, sucrose, starch, and total nonstructural carbohydrates (TNC) were also examined.

It is generally accepted that sucrose formation in plant tissues is catalyzed by sucrose phosphate synthase (SPS), whereas the breakdown of sucrose is correlated with the activities of invertase and sucrose synthase (SS) (Hubbard et al., 1991; Miron and Schaffer, 1991). There is very little, if any, information available on sucrose metabolizing enzymes in *Azolla*, especially as affected by Cr toxicity. Therefore, sucrose metabolizing enzymes under the experimental conditions were also investigated.

Materials and Methods

Preparation of Humic Substances.—Leaves of *Typha latifolia* were chopped and placed in an aquarium filled with water and allowed to decompose for approximately one year under greenhouse conditions. Naturally occurring hu-

mic substances consist predominantly of humic acid and fulvic acid (Manahan, 1994). The decomposed organic matter was collected, filtered using Whatman filter paper (pore size 11 μm), autoclaved at 20 psi, 160 °C for 15 min., then filtered again. The filtrate was then diluted with distilled water to obtain the desirable absorbance, 0.150 at 250 nm, which was observed to be the wavelength at which peak absorbance for the humic material occurred.

PLANT MATERIAL.—Cultures of *Azolla* were grown in Hoagland solution (Hoagland and Arnon, 1938), diluted 1:40 (Sela et al., 1989) at pH 6.0. The plants were placed in the growth chamber with a 14 h photoperiod and 220 μmol m^{-2} s^{-1} photosynthetic photon flux density and day/night temperatures of 25 ± 1°C. A total of 1080 plants was randomly selected and placed in 250 ml Erlenmeyer flasks (12 flasks per treatment). Each flask contained 125 ml of Hoagland solution and received fifteen selected plants. All experimental units of each treatment received Cr(VI) concentrations of 0, 1, or 2 μg ml^{-1}, with and without humic material. Chromium was added to the medium in form of $K_2Cr_2O_7$. The pH of each sample of each treatment were adjusted to 6.0.

Azolla was grown under the above conditions for 14 days with the growth media being changed at the end of the first week. At the end of the second week of treatment, six randomly selected samples of each treatment were used for dry weight and nonstructural carbohydrates. The other six samples of each treatment were used for testing of other parameters.

GROWTH DETERMINATION.—The plants of each treatment were oven dried for 2 h at 80°C in an effort to stop enzyme action, then dried for an additional 48 h at 70°C. Dry weights were recorded and the samples stored at −20°C for subsequent determination of carbohydrates.

CHLOROPHYLL AND CAROTENOID DETERMINATION.—A sample of 0.05 mg plant material was placed in a 10 ml vial of N,N-dimethylformamide (DMF) and incubated in the dark for 36 h at 4°C in order to extract chlorophyll. Chlorophyll *a* and *b* content were determined spectrophotometrically by the method of Inskeep and Bloom (1985). Carotenoid content was determined spectrophotometrically from the N,N-DMF extraction, and total concentration was calculated using the formula of Doong et al. (1993).

ANTHOCYANIN DETERMINATION.—A sample of ground plant material, approximately 0.10 g fresh weight, was homogenized in 5 ml methanol containing 1% HCl (v/v) for 2 min on ice. The homogenate was filtered and absorbance of the extract was determined spectrophotometrically by the method of Mancinelli (1990).

CARBOHYDRATE DETERMINATION.—Carbohydrate analysis of the plant samples was conducted following a slightly modified procedure from that outlined by Chatterton et al. (1987). A portion of known weight of each sample of *Azolla* was analyzed for soluble sugars, starch, and total nonstructural carbohydrates (TNC).

To determine soluble sugar concentration, dried samples were ground to a

fine powder and extracted with 5 ml 95% ethanol at 90°C for 30 min. The samples were then centrifuged at 4,000× g for 10 min and the supernatant collected. Another 5 ml 95% ethanol was added to the precipitate and remaining soluble sugars were collected as above. The supernatants were combined and used to determine total soluble sugars following the method of Dubois et al. (1956). A 0.1 ml aliquot from each of the combined ethanol extractions samples were set aside for sucrose determination following the procedure described by Van Handel (1968).

The residue remaining after ethanol extraction was used for starch determination. The residue was resuspended in 2 ml of distilled water and then placed in the hot water bath at 90°C for 1 h to gelatinize the amylopectin. Following cooling, 5 ml of 0.2M acetate buffer (pH 4.6) containing 0.5% (w/v) porcine pancreatic alpha amylase and 2% (w/v) *Rhizopus* mold amyloglucoside were added to the residue. The samples were incubated at 25°C for 1 h and 55°C for 24 h. The following days samples were centrifuged at 10,000× g for 15 min and the supernatant was collected. Five ml of water were added to the precipitate and the samples were placed in a water bath at 60°C for 10 min and recentrifuged. The combined supernatants from the two water extracts were used for glucose determination, as for ethanol extracts to determine starch concentration. The remaining residue was hydrolyzed with 5 ml of 0.6N HCl at 80°C for 1 h. Samples were centrifuged and supernatant collected and glucose was measured as in above in order to determine the residual carbohydrate in the samples. Total nonstructural carbohydrate concentration was obtained by combining the concentration of soluble sugar, starch, and the results from the HCl hydrolysis together (Chatterton et al., 1987).

ENZYME EXTRACTION.—Approximately 0.5–1.0 g fresh weight of the plants was homogenized in 10–15 ml of extraction medium containing: 50 mM Hepes-NaOH buffer (pH 7.5), 0.5 mM $MgCl_2 \cdot H_2O$, 1 mM Na Ethylenediaminetetraacetic acid (EDTA), 2 mM diethyldithio- carbamic acid (DIECA), 2.5 mM DL Dithiothreitol (DTT), 2% (w/v) insoluble Polyvinylpyrrolidone (PVP), according to the method of Hubbard et al. (1989). The extract was centrifuged for 30 min at 18,000× g. The supernatants were dialyzed for 16 h at 4°C against 25 mM Hepes-NaOH (pH 7.5).

SPS AND SS ASSAY.—A 140 µl aliquot of tissue extract was incubated at 37°C for 15 min with an equal volume of a mixture containing 15 mM UDP-glucose, 15 mM fructose 6-P, 5 mM $MgCl_2 \cdot 6H_2O$, 5 mM $Na_2MoO_4 \cdot 2H_2O$ and 50 mM Hepes-NaOH buffer (pH 7.5) for sucrose phosphate synthase assay. For sucrose synthase assays, the fructose 6-P was replaced by fructose. Reactions were terminated by the addition of 70 µl of 1N NaOH. Sucrose concentrations were determined following the method of Van Handel (1968) and compared to appropriate sucrose standards.

INVERTASE ACTIVITY.—Invertase activity was determined by measuring reducing sugars formed from sucrose hydrolysis by the Somogyi method as modified by Nelson (1944). Invertase was assayed at 30°C in digests containing 0.5 ml

TABLE 1. Interactive effects of humic substances and different concentrations of chromium (mg l^{-1}) on *Azolla caroliniana* growth. Humic material absorption was 0.150 at 250 nm. Means followed by the same letter in each column are not significantly different based on the LSD test (P = 0.05).

Treatment	Plant dry weight (mg)
Control (without humic and Cr)	17.95a
Humic	15.32b
1.0 Cr with humic	12.90c
1.0 Cr without humic	12.11c
2.0 Cr with humic	11.83cd
2.0 Cr without humic	10.83d

enzyme solution and 0.5 ml acetate buffer (0.02M, pH 7) containing 5% (w/v) sucrose. The assay was performed for 20 min. One half ml of the Nelson-Somogyi copper reagent was added to terminate the reaction, and the tubes were immediately capped and heated for 30 min in a boiling water bath. After cooling, 0.5 ml of the arseno-molybdate reagent was added and followed by 3.5–4 ml of H_2O. The absorbance was read at 660 nm and compared to the appropriate concentration of glucose standard.

STATISTICAL ANALYSIS.—This experiment was repeated twice and analyzed statistically as a randomized complete design (Steel and Torrie, 1980). This design ensured that observed differences in plants' performances were due to treatments, rather than variations among blocks (replicate series conducted at different times). Mean separations for the values that showed significant F values (P = 0.05) of the ANOVA analysis were based on the least significant difference (LSD) test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The presence of the chromium in the growth media caused a significant reduction in *Azolla* growth (Table 1). This decrease in growth of Cr(VI)-treated plants may be due in part to a reduction in nutrient uptake. Sela et al. (1989) reported that Cr(VI) exposure influenced a decrease in the essential nutrients Ca, Mg, and K. It also was evident that the growth decreased significantly with an increase in chromium concentration. Similar results were observed by several investigators, most recently by Gaur et al. (1994).

The presence of the humic substances appeared to reduce chromium toxicity to *Azolla,* at both Cr(VI) concentrations (1 and 2 mg l^{-1}), which were shown respectively an 8.9 and 9.2% growth increase in comparison to that obtained of plants grown at the same Cr(VI) concentration in the absence of humic substances. De Filippis and Palaghy (1994) suggested that bioaccumulation of any metal is a function of its ionic level in the growth media. The ionic level of the Cr(VI) was probably reduced by binding to the humic substances, thus limiting its availability to the plant. Stackhouse and Benson (1988) found that the presence of 5.0 and 50 mg l^{-1} humic acid reduced the concentration of free chromium in the media. *Azolla* growth was significantly reduced by the

TABLE 2. Interactive effects of humic substances and different concentrations of chromium (mg l^{-1}) on chlorophyll *a* (Chl*a*) and chlorophyll *b* (Chl*b*) concentrations in *Azolla caroliniana*. Means followed by the same letter in each column are not significantly different based on the LSD test (P = 0.05).

Treatment	mg g^{-1} fresh weight		
	Chl*a*	Chl*b*	Chl*a*:Chl*b*
Control (without humic and Cr)	9.78a	8.85a	1.11a
Humic	9.96a	9.84a	1.06a
1.0 Cr with humic	5.09b	2.89b	1.77b
1.0 Cr without humic	3.52c	2.06b	1.73b
2.0 Cr with humic	5.31b	2.86b	1.87c
2.0 Cr without humic	3.42c	2.03b	1.70b

presence of the humic substances in the absence of Cr(VI) (Table 1). This indicates that although humic substances reduced the toxicity of the Cr(VI) to *Azolla* growth, probably through chelation, they may also exhibit an inhibitory effect on *Azolla* growth, possibly through chelating essential nutrients.

The concentration of chlorophyll *a* and *b* of *Azolla* declined significantly in the presence of Cr(VI) (Table 2). This finding was in marked contrast to that reported previously for *Azolla,* in which Sarkar and Jana (1986) found an insignificant reduction in chlorophyll concentration at 2 and 5 mg l^{-1} Cr. However, Bassi et al. (1990) showed that a significant reduction in chlorophyll concentration of *Lemna minor* was obtained with Cr(VI) concentrations ranging from 0.1 to 10 μg l^{-1}. In the same investigation, a decrease in the chlorophyll concentration in *Pistia stratiotes* L. (Araceae) was shown at Cr(VI) concentrations of 1 to 20 μg l^{-1}. Cr(VI)-induced reduction in chlorophyll concentration may be due to swelling of the chloroplast or thylakoid disarrangement (Bassi et al., 1990) or an increase in chlorophyllase activity (Sen *et al.* 1987).

Humic substances once again seemed to influence a significant reduction in the toxic effects of Cr(VI). Humic substances reduced the effect of Cr(VI) to *Azolla* chlorophyll *a,* but exhibited no significant influence on Cr(VI) effect on chlorophyll *b* (Table 2). In the absence of Cr(VI), humic substances had no influence on chlorophyll *a* or chlorophyll *b*. The ratio of chlorophyll *a* to chlorophyll *b* was significantly increased in the presence of the Cr(VI) (Table 2). Furthermore, the presence of humic substances with Cr(VI) resulted in greater increase in the ratio of chlorophyll *a* to chlorophyll *b*. This result indicates that chlorophyll *b* was more susceptible than chlorophyll *a* to the presence of Cr(VI) with or without humic substances in the growth media.

Carotenoid concentrations were not affected by the presence of Cr(VI) with or without humic substances (Table 3). However, a 54.2% increase in the carotenoid concentration was obtained in plants grown in growth media containing humic substances without Cr(VI). Anthocyanin concentration increased in response to Cr(VI) exposure (Table 3), both 1 and 2 mg l^{-1} Cr(VI) resulted in a significant increase in comparison to controls. However, anthocyanin concentration was unchanged with increasing Cr(VI) concentration. Humic substances in the absence of Cr(VI) also influenced a significant in-

TABLE 3. Interactive effects of humic substances and different concentrations of chromium (mg l^{-1}) on concentrations of carotenoids and anthocyanins in *Azolla caroliniana*. Humic material absorption was 0.150 at 250 nm. Means followed by the same letter in each column are not significantly different based on the LSD test (P = 0.05).

Treatment	μg g^{-1} fresh weight	
	Carotenoids	Anthocyanins
Control (without humic and Cr)	64.42a	5.85a
Humic	99.36b	6.10b
1.0 Cr with humic	72.78a	6.70c
1.0 Cr without humic	61.11a	7.58d
2.0 Cr with humic	74.62a	6.69c
2.0 Cr without humic	60.12a	7.25d

crease in the anthocyanin concentration. The increase in anthocyanin concentration occurred in response to the presence of humic substances in the growth media even in the absence of Cr(VI). This conclusion also was supported by Doong et al. (1993), who concluded that anthocyanins are produced by most aquatic plants in response to stress factors, such as high light intensity, high temperature, or nutritional limitations, and can be used as a stress indicator. Humic substances appeared to reduce anthocyanin concentration in those treatments receiving Cr(VI) in comparison to the same Cr(VI) concentration in the absence of humic substances.

Accumulation of total nonstructural carbohydrates increased significantly in *Azolla* grown in the presence of Cr(VI) at both concentrations (Table 4). These results indicate that Cr(VI) was probably less inhibitory to *Azolla* photosynthesis than it was to overall growth, thereby confirming the observations made by Schroll (1978) while investigating Cr toxicity to the alga *Chlorella pyrenoidosa* Chick. In the present study, the observed increase in carbohydrate accumulation by plants treated with Cr(VI) might be due to a reduction in carbohydrate utilization. Azcon-Bieto (1983) reported that lower rates of carbon assimilation and a decrease in yield were associated with carbohydrate accumulation in many plant species. A reduction in plant respiration in response to Cr(VI) exposure was reported by Bassi et al. (1990). Cr(VI) was also found to cause mitochondrial damage (Fasulo et al., 1983). Humic substances were shown to influence an insignificant increase in TNC accumulation in comparison to that of the control plants in the absence of Cr(VI), and a significant reduction in TNC accumulation in comparison to that of plants when Cr(VI) was present. The same result was obtained for starch (Table 4).

The presence of humic substances and Cr(VI), singly or combined, resulted in an increase in the sucrose concentration (Table 4). However, this increase was insignificant in the presence of humic substances with or without 1 mg l^{-1} Cr(VI). Increased Cr(VI) concentrations caused an elevation in sucrose accumulation. Treatments receiving both humic substances and Cr(VI) exhibited a slight and insignificant decrease in sucrose accumulation in comparison to that obtained of the same Cr(VI) treatment in the absence of humic substances.

TABLE 4. Interactive effects of humic substances and different concentrations of chromium (mg l^{-1}) on sucrose, starch, and accumulation of total nonstructural carbohydrates (TNC) in *Azolla caroliniana*. Humic material absorption was 0.150 at 250 nm. Means followed by the same letter in each column are not significantly different based on the LSD test (P = 0.05).

Treatment	mg g^{-1} dry weight		
	Sucrose	Starch	TNC
Control (without humic and Cr)	15.28a	276.77a	349.45a
Humic	17.77ab	297.83a	382.45a
1.0 Cr with humic	19.71abc	376.84b	466.17b
1.0 Cr without humic	21.51bc	379.05b	480.16b
2.0 Cr with humic	22.83bc	388.12b	496.03b
2.0 Cr without humic	23.69c	392.08b	503.80b

These higher concentrations of sucrose are not indicative of a higher rate of synthesis, but may result from increased accumulation in the vacuole (Gieger et al. 1983).

The activity of SS was significantly higher in *Azolla* exposed to humic substances (Table 5). Humic substances in the presence of Cr(VI) seemed to influence a slight insignificant increase in SS activity in comparison to that obtained of the same Cr(VI) treatment in the absence of the humic. With the exception of the Cr(VI) concentration of 1 mg l^{-1} without humic, the activity of SS was significantly higher in *Azolla* grown in the presence of Cr(VI). However, there were insignificant differences in SS activity between the Cr(VI) concentrations. The increase in SS activity in *Azolla* grown in the presence of Cr(VI) with or without the association of humic substances could be used to explain the fact that starch accumulation increased with these treatments. Wang et al. (1993) reported that SS was the predominant enzyme catalyzing the first reaction of starch formation that results in increasing glucose concentrations through sucrose hydrolysis. Furthermore, Doehlert (1990) reported that increased SS activity was associated with starch synthesis.

On the other hand, invertase activity was reduced in the presence of the humic substances, with and without Cr(VI). However, this reduction in activity was insignificant at the Cr(VI) concentration of 1 mg l^{-1}. The observed reduction in invertase activity in the presence of humic substances might be due to a formation of an enzyme-humic substance complex, which might result in reducing the enzyme's activity. Wetzel (1993) reported humic substances extracted from decomposed *Typha* were shown to markedly suppress phosphatase activity of planktonic and attached algae, bacteria, and vascular aquatic plants. In the absence of the humic substances, Cr(VI) influenced a significant increase in invertase activity in *Azolla* grown at both concentrations. Wang et al. (1993) reported a constant positive relationship between SS activity, but not that of invertase, and starch content. As with invertase, humic substances also resulted in a reduced activity of SPS (Table 5). A Cr(VI) concentration of 1 mg l^{-1} influenced an insignificant increase in SPS activity. However, SPS activity of *Azolla* grown in a media containing 2 mg l^{-1} Cr(VI) with or without

TABLE 5. Interactive effects of humic substances and different concentrations of chromium (mg l^{-1}) on the activity of sucrose metabolizing enzymes in *Azolla caroliniana*: sucrose synthase (SS); sucrose phosphate synthase (SPS); and invertase. Invertase activity measurements represent mg glucose g^{-1} fr. wt. $min.^{-1}$, whereas SS and SPS activities measurements represent mg sucrose g^{-1} fr. wt. $min.^{-1}$. Humic material absorption was 0.150 at 250 nm. Means followed by the same letter in each column are not significantly different based on the LSD test (P = 0.05).

Treatment	mg g^{-1} fresh weight $min.^{-1}$		
	SS	SPS	Invertase
Control (without humic and Cr)	0.67a	0.82a	55.37a
Humic	0.41b	0.57b	35.35b
1.0 Cr with humic	0.58c	0.79a	53.86a
1.0 Cr without humic	0.62ac	0.85a	60.72c
2.0 Cr with humic	0.57c	0.52b	48.96d
2.0 Cr without humic	0.58c	0.70ab	58.96c

humic substances was significantly reduced. This result indicated that the influence of Cr(VI) on SPS activity is related to Cr(VI) concentration. Nomura and Akazawa (1974) reported that metal ions including Co^{+2}, Fe^{+2}, $Fe,^{+3}$, Mg^{+2}, and Mn^{+2} stimulated the activity of SPS at relatively low concentrations. In this investigation, the increase in sucrose concentration in the presence of humic substances and with increased Cr(VI) concentrations was not correlated with the increase in SPS activity (Tables 4 and 5). Sucrose was found to be a potential inhibitor of SPS in several studies (Rufty and Huber, 1983; Huber, 1981; Salerno and Pontis, 1978). Similar to our findings, Huber and Israel (1982) reported a negative relationship between the activity of SPS and starch accumulation in leaf extracts of *Glycine max* L. (Fabaceae) exposed to a variety of environmental and nutritional conditions.

In conclusion, results from the present study indicate that reductions in growth and chlorophyll production and increases in anthocyanins and carbohydrate accumulation in *Azolla* were not as great in those treatments receiving humic substances in addition to Cr(VI) in comparison to the treatments that received Cr(VI) in the absence of humic substances. These results suggest that the presence of humic substances decreased the toxic effects of Cr(VI). In addition, Cr(VI) caused an increase in the activities of sucrose metabolizing enzymes, with the exception of SPS activity which was decreased at 2 mg l^{-1}. Humic substances caused an increase in SS activity in the absence of Cr(VI), but decreased SPS and invertase both with and without Cr(VI). The greatest reduction in the activities of SPS and invertase occurred when humic substances were present without Cr(VI).

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